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Effective degradation of organic pollutants in aqueous media by microbial strains isolated from soil of a contaminated industrial site

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Abstract

Background: Bioremediation is a low-cost technology, whose efficacy is often enhanced with preliminary mild physical–chemical remediation methods. A further advantage of bioremediation resides in its eco-compatibility and, thus, sustainability. Two autochthonous microbial strains, *Methylobacterium populi* VP2 and *Aspergillus sydowii* VP4, were isolated from a soil of a highly contaminated industrial site and used to degrade the aqueous extract of contaminants (AEC) obtained from the same polluted soil.

Results: The AEC incubation with both strains produced a significant removal of most organic pollutants, although the degradation capacity decreased with increasing AEC concentration in the minimal selective liquid medium (MSML) of the experiments. At 30 % of AEC, *M. populi* VP2 determined the removal of most pollutants and the appearance of new products due to oxidation and enzymatic degradation. Incubation of *A. sydowii* VP4 at the same AEC concentration in MSML removed the same pollutants but also the derived degradation products. Our results showed that the strains isolated from a highly contaminated soil maintained the capacity to use organic contaminants as metabolic carbon in aqueous extracts from the same soil. The greater biodegradation efficiency of the fungal strain in comparison to *M. populi* VP2 may be caused by a modification of the *A. sydowii* VP4 cell surface that increases cell permeability to hydrophobic compounds and thus enhances the extent of pollutants degradation.

Conclusions: This work indicates that two specific strains, *M. populi* VP2 and *A. sydowii* VP4, isolated from the soil of a highly contaminated site are not only useful in the treatment of leaching polluted waters but may also be used in bioaugmentation practices during remediation of contaminated soils.

Keywords: Autochthonous microbial strains, Organic pollutants, Biodegradation in aqueous soil extracts of contaminants, Bioaugmentation

Background

Waste management policies are often inadequate to keep the pace with increased industrialization and lead to a significant environmental degradation by toxic and hazardous pollutants. Several contaminants, such as heavy metals, radionuclides, and recalcitrant organic compounds, including pesticides, dyes, Polycyclic Aromatic Hydrocarbons (PAHs) and PoliChlorinated Bihenyls (PCBs), may persistently accumulate in soils and sediments, thus potentially menacing health security and environment quality. This risk is magnified by the bioconcentration phenomenon of toxic, carcinogenic and mutagenic compounds throughout the trophic chain [1]. An essential priority is the remediation of industrial soils contaminated by persistent organic pollutants, since they represent a significant hazard to terrestrial and aquatic ecosystems and food security [2].

Traditional chemical and/or physical methods generally applied to eliminate pollutants from soil have inherent

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drawbacks such as high operational costs and frequent production of secondary pollutants [3] and references therein. Despite its long-time requirement, bioremediation is an alternative low-cost technology, whose efficacy is often enhanced with preliminary mild physical–chemical remediation methods [4, 5]. A further advantage of bioremediation resides in its eco-compatibility and, thus, sustainability.

Although several microbial strains naturally present in contaminated soils and aquifers are capable of biodegrading organic pollutants, they may not display the appropriate metabolic capacity towards target pollutants due to overall toxicity of contaminants [6]. In fact, biodegradation of pollutants relies on integrated activities of enzymes, cells, and microorganisms communities, whose regulation and kinetics are highly specific, though also greatly variable in space and time. Since biodegradation depends on environmental factors, including nutrients levels, availability of substrates, predation, population density, contaminant concentration, pre-exposure, and bioavailability [7], active microbial communities must often be either sustained with either metabolic substrates or specifically potent external communities.

The enhanced activation of native soil microorganisms by nutrients addition is commonly referred to as biostimulation, while bioaugmentation regards the practice of spreading soil with pre-grown microbial cultures, which are selected to perform a specific remediation task in a given environment [8, 9]. Within bioaugmentation, critical environmental factors are both predation by protists, and competition by the autochthonous microorganisms, and risk to jeopardize the active survival of the introduced exogenous degraders and consequently negatively affect bioremediation [10].

The aim of this study was to assess the ability of selected autochthonous microbial strains, previously isolated from a highly contaminated industrial soil, to degrade the different classes of recalcitrant organic contaminants when added aqueous extracts of contaminants (AEC) obtained from the same soil. The validation of the activity of microbial strains used in this study may support the development of more efficient bioaugmentation processes to be extended to bioremediation of polluted soils.

Methods

Soil samples

Samples that mainly included the arable soil layer (0–30 cm depth) were collected from the site of ACNA (Aziende Chimiche Nazionali Associate), an industrial area of Cengio (near Savona) in the Northern Italy. The site is extremely polluted due to irregular disposal of organic and inorganic contaminants on surface and lower soil horizons since 1882. The serious contamination of surrounding soils and waters has led the ACNA site to be included in the list of national priorities for environmental remediation. The chemical and physical properties of the studied soils are reported elsewhere [5].

Solubilization of soil contaminants in aqueous solutions

Contaminants were extracted from the ACNA soil by refluxing 30 g of soil in a Soxhlet equipment for 48 h with 225 mL of an acetone/*n*-hexane (1:1) mixture. The organic extract was first dried in a roto-evaporator at 40 °C and redissolved in 5 mL of acetone and 145 mL of ultrapure water (final volume of 150 mL that constituted the AEC), in order to obtain an aqueous extract of contaminants (AEC). The content of organic pollutant in AEC was determined before and after incubation with selected microbial strains. Briefly, the AEC samples were subjected to solid phase extraction (SPE) by adsorption on Bond-Elute C-18 cartridges (500 mg/3 mL by Varian), followed by elution with 10 mL of *n*-hexane, 10 mL of diethyl ether and 10 mL of acetone, in this order. The eluted solution was dried and redissolved with 1 mL of CH_2Cl_2, 1 mL of a 100 μg/mL octafluoronaphtalene solution in CH_2Cl_2 (internal standard), and analysed by Gas Chromatography–Mass Spectrometry (GC–MS). The concentration of contaminants identified by GC–MS analysis was related to that octafluoronaphtalene whose detection limit was 5 μg/mL.

Microbial strains

The two microbial strains used for this study and isolated from the contaminated soil of ACNA site were: (1) the plant growth promoting *Methylobacterium populi* VP2 capable of producing biofilms and recognized to alleviate phenanthrene stress in tomato seeds [11], and (2) a fungal strain that was identified by the sequencing ITS region, including ITS1-5.8S rDNA-ITS2. For the latter strain, total genomic DNA was extracted and purified by InstaGene™ Matrix (Bio-Rad Laboratories, Milan, Italy) according to the supplier's recommendations. Approximately 50 ng of DNA was used as template for PCR assay. The synthetic oligonucleotide primers, ITS 1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS 4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) were used to amplify the ITS region [12]. The PCR mixture and conditions were prepared and conducted as reported by Alfonzo [13] and Palomba [14], respectively. The PCR products were first verified by agarose (1.5 % wt/vol) gel electrophoresis, at 100 V for 1 h, purified by using a QIAquick gel extraction kit (Qiagen S.p.A, Milan, Italy), and then sequenced. The DNA sequences were determined and analysed as previously reported [15], and compared to the GenBank nucleotide data library using the Blast software at the National Centre of Biotechnology

Information website (http://www.ncbi.nlm.nih.gov/Blast.cgi) [16]. The obtained sequence was deposited in the GenBank database under accession number KF955559.

The sequence of the ITS region of the fungal strain VP4 showed a 100 % identity with *Aspergillus sydowii* species using the Blast software. To confirm this result, a phylogenetic tree was also constructed that included 17 *Aspergillus* species. The results of the neighbour-joining analysis of the ITS region sequences of the 17 *Aspergillus* strains and VP4 are shown in the dendrogram of Fig. 1. The closest relative of VP4strain was *Aspergillus sydowii*.

Multiple nucleotide alignment of ITS region of the fungal strain and 17 strains belonged to the genus *Aspergillus* was carried out using the Clustal W program [17] from the MEGA version 4.0 [18]. The nucleotide sequences of type strains were retrieved by the National Centre for Biotechnology Information (NCBI—www.ncbi.nlm.nih.gov). The phylogenetic tree was inferred by using the Neighbour-Joining method with the Maximum Composite Likelihood model in MEGA4 program with bootstrap values based on 1000 replications.

Biodegradation of soil contaminants in aqueous extracts

The potential biodegradation activity of the selected microbial strains was tested. In detail, the strains were inoculated in 5 mL of a minimal selective liquid medium (MSLM) containing 2.25 g/L NaCl, 0.105 g/L KCl, 0.12 g/L $CaCl_2$, 0.05 g/L $NaHCO_3$ and 10 % (v/v) of AEC. After incubation at 28 °C for 24 h, a volume of the broth culture corresponding to 0.1 OD was used to inoculate MSLM containing different concentrations of AEC (0, 30, 40, and 50 %, v/v), as sole carbon source. All growth media contained equal concentration of basal salts (2.25 g/L NaCl, 0.105 g/L KCl, 0.12 g/L $CaCl_2$, 0.05 g/L $NaHCO_3$). Un-inoculated MSLM were used as control. The cultures were incubated in an orbital shaker at 120 rpm for 15 days at 28 °C. After incubation, samples were centrifuged for 10 min at 4000 *rpm* and the supernatant was subjected to GC–MS analysis for the determination of contaminants and their content.

Moreover, microbial enumeration was also performed by the spread plate count method using minimal selective solid medium (MSSM) with the same amount of AEC (30, 40, and 50 %, v/v) in order to ascertain their viability in the MSLM at the end of the experimentation. The plates were incubated at 28 °C for 7 days. All tests were performed in triplicate.

Gas chromatography–mass spectrometry (GC/MS) analysis

A Perkin-Elmer Autosystem XL gas-chromatograph, equipped with a Programmed-Temperature Split/

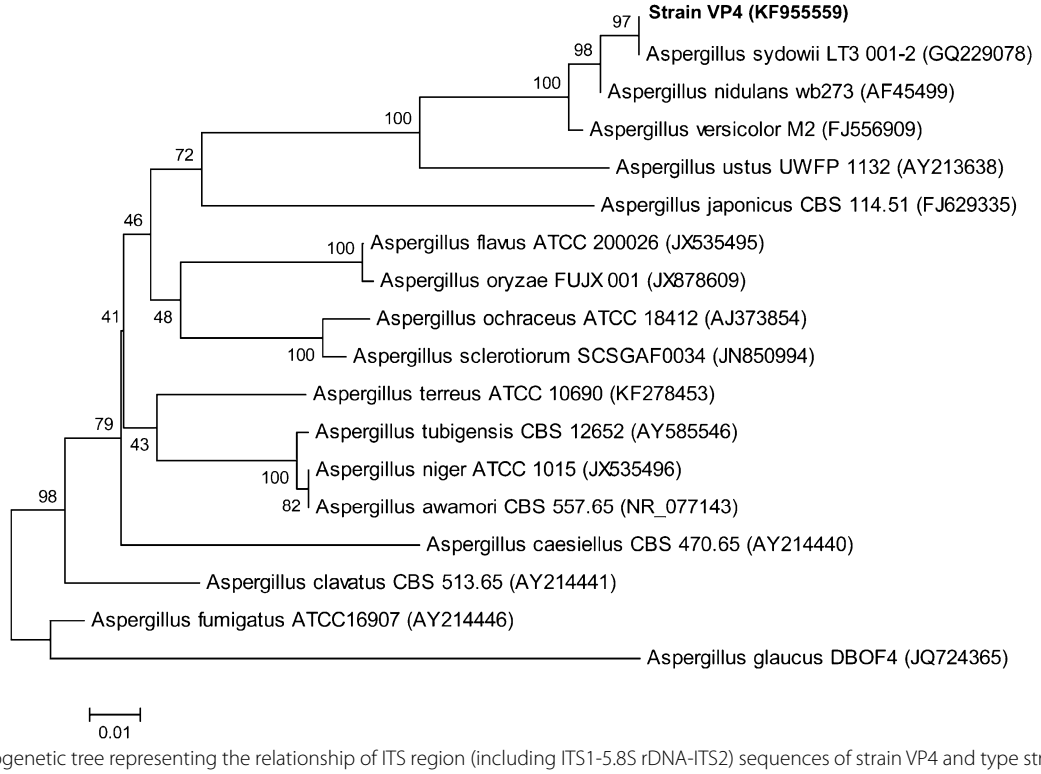

Fig. 1 Phylogenetic tree representing the relationship of ITS region (including ITS1-5.8S rDNA-ITS2) sequences of strain VP4 and type strains *Aspergillus* sequences from NCBI. Bootstrap values (expressed as percentages of 1000 replications) are given at the nodes. Sequence accession numbers used for phylogenetic analysis are shown in parentheses following the species name. The *scale bar* estimates the number of substitutions per site

Splitless injector with programmable pneumatic control kept at a constant temperature of 250 °C, a Restek Rtx-5MS capillary column (5 % diphenyl-95 % dimethylpolysiloxane, length 30 m, 0.25 mm ID, and 0.25 μm df), and a Perkin-Elmer TurboMass Gold mass-spectrometer, was used for qualitative and quantitative analysis of contaminants. The conditions used for GC analyses were the following: (1) initial temperature of 40 °C for 5 min; (2) to 250 °C at a 3 °C/min rate; 3. isothermal for 20 min. The total GC run time was 95 min. Helium was the carrier gas at 1.5 mL/min with a split-flow of 30 mL/min. The inlet-line temperature of the GC–MS system was set at 280 °C, while that of the MS source at 180 °C. A solvent delay time of 5 min was applied before acquisition of the mass spectra to prevent filament injuries. Low and high m/z limits of mass spectrometer were set at 50 and 400 amu, respectively. A NIST mass spectral library version 1.7 was used for peak identification. Each GC–MS analysis was done in duplicate and quantitative results obtained by GC–MS were weight-averaged to provide experimental error. The relative standard deviation never exceeded 4 %.

Statistical analysis

One-way ANOVA followed by Tukey's HSD post hoc for pairwise comparison of means (at $p < 0.05$) was used to assess the difference in the microbial growth. Statistical analysis was performed using SPSS 13.0 statistical software package (SPSS Inc., Cary, NC, USA).

Results and discussion

The two microbial strains used in this study were able to grow in the minimal selective liquid medium (MSLM) containing different AEC concentrations. In fact, after 15 days of incubation at 28 °C, both *M. populi* VP2 and *A. sydowii* VP4 increased over 2 log CFU/mL in the presence of AEC, reaching values of about 7.3 and 5.3 log CFU/mL, respectively (Table 1). On the contrary, the two selected strains were unable to grow in the MSLM with 0 % of AEC (Table 1). These results indicate the ability of these strains to efficiently use the organic contaminants of AEC as carbon source for their growth.

A number of contaminants present in AEC samples were identified by GC–MS analysis (Table 2), although the total sum of different compounds varied, but not linearly, with the concentration of AEC in MSLM samples. In fact, the sum of contaminants concentration was 194670, 220000, and 303160 μg g^{-1} when the AEC reached 30, 40, and 50 %, respectively, in MSLM before incubation with the selected microbial strains. The largest contaminants concentration was found for 2,5-dichlorobenzenamine and diphenylsulfone, regardless of the AEC percentage in MSLM samples (Table 2). This is in line with the type of soil pollution deriving from the historical production of dyes by the dismissed ACNA factory. In fact, dicholoranilines intermediates such as dichlorobenzenamines are commonly used in the synthesis of various herbicides, azo-dyes, and other industrial chemicals, and maintain a long-term stability and toxicity in the environment [19]. Similarly, sulphur-containing polycyclic aromatic hydrocarbons (PAHs) such as dibenzothiophene and diphenylsulfone of industrial origin are known to be recalcitrant to biodegradation and only partially and slowly mineralized, thus leaving numerous toxic byproducts in the environment [20].

The inoculation of *M. populi* VP2 and *A. sydowii* VP4 strains in MSLM with different AEC concentrations generally produced a degradation of the contaminants identified before the microbial treatment. In fact, at the AEC concentration of 30, 40, and 50 % in MSLM samples, a significant removal of most organic pollutants was detected for both microbial treatments (Fig. 2 and Table 2), thus confirming that the strains previously isolated from the ACNA soil were still capable to utilize the same organic contaminants as metabolic carbon.

Many bacterial and fungal strains are employed in bioremediation of contaminated soils, as for both techniques of biostimulation and bioaugmentation. White rot fungi are reckoned to synthesize unspecific enzymes capable to

Table 1 Microbial counts (log CFU/mL) of the strains *Methylobacterium populi* VP2 and *Aspergillus sydowii* VP4 immediately after inoculation (0 days) and after 15 days of incubation in minimal selective liquid medium (MSLM) containing 0, 30, 40, and 50 % of AEC

Microbial strain	0 days				15 days			
	MSLM with 0 % AEC	MSLM with 30 % AEC	MSLM with 40 % AEC	MSLM with 50 % AEC	MSLM with 0 % AEC	MSLM with 30 % AEC	MSLM with 40 % AEC	MSLM with 50 % AEC
Methylobacterium populi VP2	4.86 ± 0.15^{b}	4.85 ± 0.10^{b}	4.88 ± 0.09^{b}	4.83 ± 0.13^{b}	0.00 ± 0.00^{a}	7.27 ± 0.20^{c}	7.41 ± 0.12^{c}	7.33 ± 0.16^{c}
Aspergillus sydowii VP4	2.88 ± 0.20^{b}	2.83 ± 0.21^{b}	2.89 ± 0.17^{b}	2.94 ± 0.24^{b}	0.00 ± 0.00^{a}	5.44 ± 0.19^{c}	5.30 ± 0.13^{c}	5.34 ± 0.22^{c}

The values represent the mean ± SD of two replicates of three independent experiments. Different letters after values in the same row indicate significant differences ($p < 0.05$)

Table 2 Concentration (μg g^{-1}) of compounds identified by GC–MS in untreated AEC samples at three different percentages (30, 40, and 50 %) in minimal selective liquid medium (MSLM)

Number	Compound	30 %	40 %	50 %
1	3-Methyl 2-(2-oxopropyl)furan	10580	2800	0.642
2	2,5-Dichlorobenzenamine	44250	63500	89000
3	2,4-Dichlorobenzenamine	18250	25380	33000
4	Tritetracontane	3810	10400	15570
5	1,2-Benzenedicarboxylic acid, bis(2-ethylpropyl)ester	11120	2580	3500
6	Diphenylsulfone	96000	110000	132000
7	Tetradecanoic acid 10,13-dimethyl,methylester	7940	2100	7750
8	Heptadecanoic acid 16-methyl-, methylester	2720	3150	21700
	Total	*194670*	*220000*	*303160*

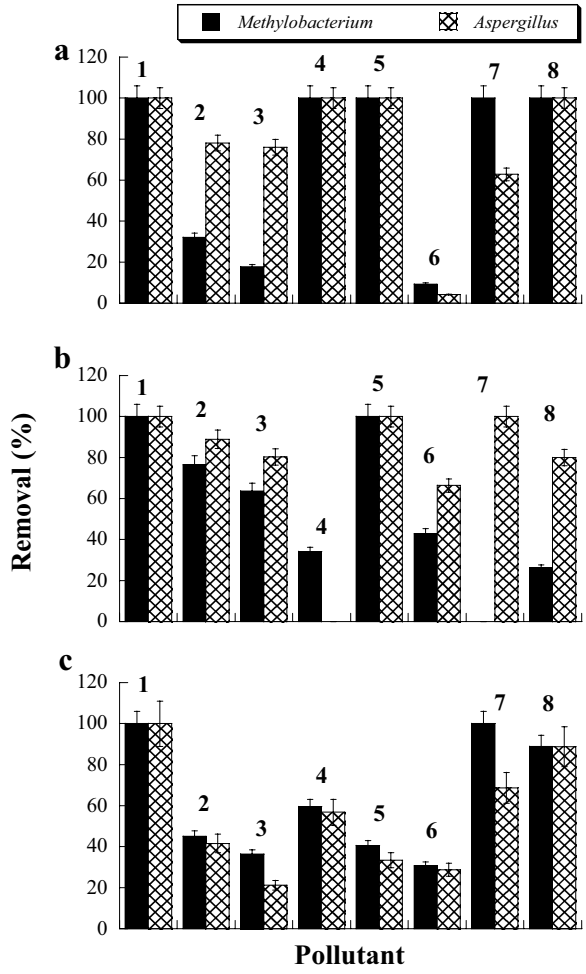

Fig. 2 Percent removal (%) of compounds identified in AEC (see Table 1) at 30 (**a**), 40 (**b**), and 50 % (**c**) concentration in MSLM samples, following incubation with *Methylobacterium* and *Aspergillus* microbial strains

degrade complex high molecular-weight and recalcitrant toxic compounds [21]. The fungi exude copiously organic acids and oxidative enzymes from their mycelia, thus contributing to biodegrade organic contaminants in soil. In particular, white-rot basidiomycetes species such as *Pleurotus ostreatus* and *Trametes versicolor* co-metabolically degrade toxic compounds, such as PAH, by a considerable production of ligninolytic enzymes [22–25]. Nevertheless, even non-ligninolytic fungi such as *Aspergillus niger* are reported to oxidize a variety of PAH, including chrysene and benzo(a)pyrene, by an intracellular cytochrome P-450 dependent oxidase system [26].

Very few studies reported the ability of *A. sydowii* species to degrade different classes of hydrocarbons. A strain of *A. sydowii*, isolated from highly contaminated soils, was recognized in in vitro assays to enable degradation of up to 81 % of total petroleum hydrocarbon (TPH), as well as five- and six-ring aromatic compounds [27]. This strain was also assayed in soil experiments to verify its efficiency to degrade contaminants. In particular, Mancera-Lopez [28] compared the efficacy of biostimulation and bioaugmentation treatments to a silty-loam soil contaminated with 60600 mg kg^{-1} of a complex mixture of total petroleum hydrocarbons (TPH), containing 40 % aliphatic hydrocarbons (AH) and 21 % polycyclic aromatic hydrocarbons (PAH). Bioaugmentation made use of *Rhizopus* sp., *Penicillium funiculosum* and *Aspergillus sydowii* strains, that had been previously isolated from two aged soils contaminated with 60600 and 500000 mg of TPH per kilogram of dried soil. These authors showed that, similarly to our findings, bioaugmentation was highly effective, since the three fungal strains enabled, respectively, the removal of 36, 30, and 17 % more PAH than for the application of simple biostimulation measures.

Moreover, there are also several studies that describe the capacity of many bacterial genera and species to mineralise and/or degrade xenobiotic environmentally toxic compounds, such as hydrocarbons. In particular, different methylotrophic bacteria are known to be potentially biodegrading agents toward different organic pollutants, such as explosive, methyl tert-butyl ether (MTBE), and polycyclic aromatic hydrocarbon (PAH) [29–31]. However, to the best of our knowledge, only the *M. populi* VP2 strain among the *Methylobacterium* species was previously reported to enable degradation of PAHs, such as phenanthrene [11].

Despite the general efficacy of the bioaugmentation treatment of AEC, here we found that the removal of contaminants by microbial degradation becomes progressively less efficient with increasing AEC concentration in MSLM (Fig. 2; Table 2). At the smallest AEC concentration (30 %), both microbial strains totally removed 3methyl 2-(2-oxopropyl)furan (1), tritetracontane (4), 1,2 benzenedicarboxylic acid, bis(2-methylpropyl)ester (5), and heptadecanoic acid 16-methyl-, methylester (8). At the same AEC concentration, *A. sydowii* VP4 was able to remove up to 78 and 76 % of 2,5-dichlorobenzenamine (2) and its 2,4 dichlorobenzenamine (3) isomer, respectively, whereas *M. populi* VP2 removed only 32 and 18 % of the two compounds, respectively.

Conversely, diphenylsulfone (6) was hardly degraded by both *M. populi* VP2 and *A.* sydowii VP4 microbial strains at 30 % AEC concentration (Fig. 2a). Surprisingly, although the concentration of diphenylsulfone (6) increased in AEC from 110000 and 132000 μg g^{-1} in the MSLM at 40 and 50 % AEC concentration, respectively (Table 2), its degradation reached 54 and 30 % of the original content at the AEC concentration in MSLM, respectively (Fig. 2b, c). It is also interesting to note that tritetracontane (4) was completely degraded by both microbial strains at the smallest AEC concentration in MSML, whereas none or modest removal was observed at greater AEC concentrations (Fig. 2).

These results suggest that the microbial strains effectiveness in bioaugmentation is dependent on the pollutants concentration that regulates their bioaccessiblity within complex associations [32]. In fact, a number of studies report that the bioaccessible low molecular weight alkanes are rapidly degraded by microorganisms [33, 34]. Their degradation involves the sequential oxidation to an alcohol, an aldehyde, and a fatty acid, that is in turn reduced in chain length by progressive decarboxylation through the β-oxidation pathway. About 95 % of low molecular weight alkanes (<C30–C40) were reported to be completely mineralized in few months under the action of a strain of *Bacillus* sp. and fungal species such as *Aspergillus* spp. and *Fusarium* sp., whereas the >C40 alkanes were not mineralized [35]. Again, the environmental persistence of the larger alkanes should be attributed to the hydrophobic effect [36] that determines their strong association in large aggregates, with a consequent very poor bioaccessibility.

The removal of contaminants from AEC upon microbial treatment and the appearance of degradation products can be followed from GC–MS chromatograms. In Fig. 3 we report the chromatographic profiles of detected pollutants in MSML samples with 30 % of AEC, before and after the incubation with microbial strains, while Table 3 lists the detected compounds name and relative retention times in chromatograms.

The chromatographic signals corresponding to 3-methyl 2-(2-oxopropyl)furan (1), tritetracontane (4),

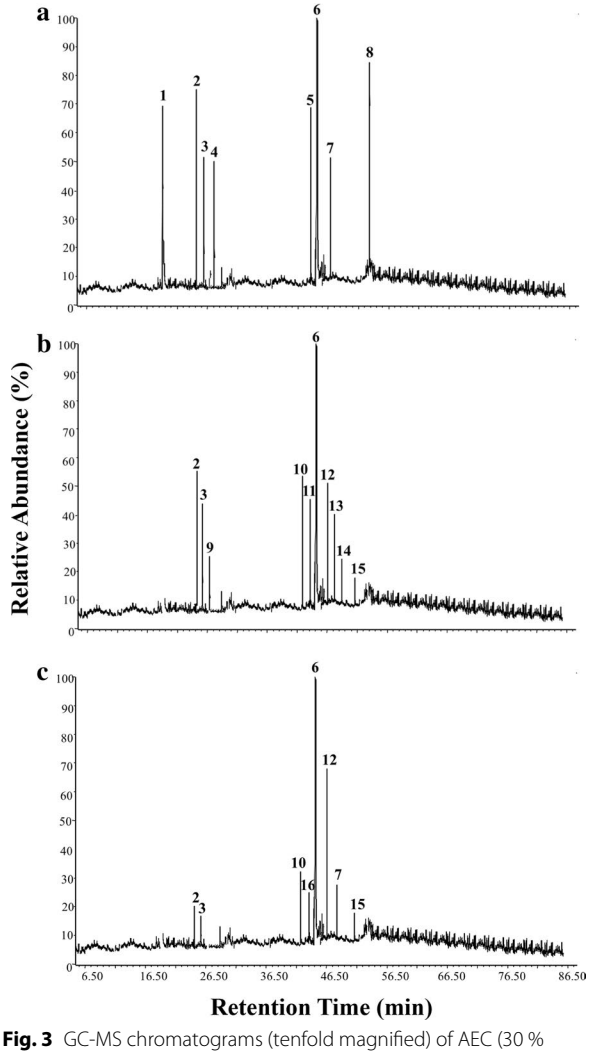

Fig. 3 GC-MS chromatograms (tenfold magnified) of AEC (30 % concentration in MSLM) before (**a**) and after treatment with selected *Methylobacterium* (**b**) and *Aspergillus* (**c**) microbial strains

Table 3 Compounds identified at different retention times (RT) by GC–MS before and after treatment of AEC samples (30 % in the minimal selective liquid medium) with *Methylobacterium* and *Aspergillus* selected microbial strain

RT (min)	Number	Compound	Untreated AEC	*Methylobacterium* strain	*Aspergillus* strain
19.45	1	3 Methyl 2-(2-oxopropyl)furan	F	NF	NF
24.79	2	2,5 Dichlorobenzenamine	F	F	F
25.23	3	2,4 Dichlorobenzenamine	F	F	F
27.24	4	Tritetracontane	F	NF	NF
44.62	5	1,2 Benzenedicarboxylic acid, bis(2-methylpropyl)ester	F	NF	NF
46.40	6	Diphenylsulfone	F	F	F
46.89	7	Tetradecanoic acid 10,13-dimethyl,methylester	F	NF	F
53.11	8	Heptadecanoic acid 16-methyl-, methylester	F	NF	NF
26.50	9	Benzenamine 4-chloro-2,5 dimethoxy	NF	F	NF
42.51	10	Phenol, 3,5-dimethoxy	NF	F	F
43.64	11	Diphenylsulfoxide	NF	F	NF
46.55	12	[1,2]pyrazine-1,4,dione,hexahydro-3-(2-methylpropyl)- pyrrole	NF	F	F
47.52	13	9,10 Anthracenedione	NF	F	NF
49.50	14	2H,8H-benzo[1,2-B:5,4-B]dipyran-2-one, 8,8-dimethyl	NF	F	NF
51.45	15	N-methyl-N-phenyl-benzenesulfonamide	NF	F	F
43.68	16	[(3-Methyl-2 butenyl)oxy]-benzene	NF	NF	F

F found, *NF* not found

1,2 benzene dicarboxylic acid, bis(2-methylpropyl)ester (5), tetradecanoic acid 10,13-dimethyl-,methylester (7), and heptadecanoic acid,16-methyl-,methylester (8) were no more visible in AEC samples after treatment with *M. populi* VP2. On the other hand, the incubation with *A. sydowii* VP4 strain produced the disappearance of the same peaks, except for that of tetradecanoic acid (7), that was still visible, though strongly reduced.

New signals appeared in the AEC sample inoculated with *M. populi* VP2 (Fig. 3b), which were identified as benzenamine 4-chloro-2,5-dimethoxy (9), phenol, 3,5-dimethoxy (10), diphenylsulfoxide (11), [1, 2] pyrazine-1,4,dione,hexahydro-3-(2-methylpropyl)-pyrrole (12), 9,10-anthracenedione (13), 2H,8H-benzo[1,2-B:5,4-B]dipyran-2-one, 8,8-dimethyl (14), and *N*-methyl-*N*-phenyl-benzenesulfonamide (15) (Table 3). These compounds were the oxidation derivatives of the enzymatic degradation induced by the microbial strains.

The contaminants degradation capacity of the *A. sydowii* VP4 fungal strain inoculated in MSML at 30 % AEC concentration seemed to be more effective than that of *M. populi* VP2 (Fig. 2c). In fact, only fewer peaks were visible in the relative chromatogram and were assigned to benzenamine 4-chloro-2,5-dimethoxy (9), phenol, 3,5-dimethoxy (10), [1, 2] pyrazine-1,4,dione,hexahydro-3-(2-methylpropyl)-pyrrole (12) and *N*-methyl-*N*-phenyl-benzenesulfonamide (15), although a new signal became visible only in this chromatogram, and was identified as [(3-methyl-2 butenyl)oxy]-benzene (16).

The greater biodegradation efficiency of *A. sydowii* VP4 in comparison to *M. populi* VP2 is to be accounted to their different processes of interactions with exogeneous compounds. The large microbial capacity to degrade hydrocarbons [37] is assigned to the bacterial efficiency in their exploitation as metabolic carbon, through the production of emulsifying biofilms that in turn increase the solubility in water of these hydrophobic compounds and thus their bioaccessibility. On the other hand, fungi are capable to degrade hydrophobic pollutants by modifying their cell surface hydrophobicity (CSH). It has been shown that the mainly hydrophilic cell surface of *M. populi* VP2 increased its CSH values by 12 % in the presence of phenanthrene (PHE), thereby becoming more accessible to the uptake of this compound [11]. The surface cell modification of the *A. sydowii* VP4 strain may account for an increased cell permeability to hydrophobic compounds, and, thus, explain the larger pollutants degradation in AEC of this study in respect to *M. populi* VP2.

Conclusions

In this study, we proved that the selected microbial strains *M. populi* VP2 and *A. sydowii* VP4, isolated from a highly contaminated industrial soil, were effective in the bioaugmentation treatment of aqueous contaminated extracts obtained from the same polluted soil. Moreover, the fungal strain *A. sydowii* VP4 was found to be more effective than the bacterial strain *M. populi* VP2 in the degradation of pollutants present in the aqueous extracts;

this behaviour may be accounted to a modification of the *A. sydowii* VP4 cell surface that increases cell permeability to hydrophobic compounds and thus enhances the extent of pollutants degradation. The generally high transformation efficiency of the organic contaminants demonstrated that both the selected microbial strains are good candidates for future applications in soil bioremediation processes.

Authors' contributions

FS, AN, VV, OP and AP participated in the isolation of microbial strains, biodegradation studies and drafted the manuscript. FS and AN carried out biodegradation studies and chemical analyses. VV and OP carried out the microbiological studies. AP participated in design and coordination of the study and drafted the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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